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THE PRIMARY STRUCTURE OF SILLUCIN, AN ANTIMICROBIAL PEPTIDE FROM *MUCOR PUSILLUS*

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1. Introduction

It has been reported that the antimicrobial activity of the thermophilic fungus, *Mucor pusillus*, resides in the unique peptide produced by this organism in liquid culture [1]. This peptide sillucin is active only against gram-positive bacteria and its apparent site of action is at the level of RNA metabolism [2,3].

The antibiotic sillucin has been described in detail [3], and is a 30 residue peptide containing 4 disulfide bridges, no methionine, phenylalanine or histidine residues. A family of related peptides is also synthesized by different strains of *M. miehei*, another thermophilic species in the genus *Mucor* [3].

As a beginning to the elucidation of the structure–function correlations of these unique fungal antibiotic peptides, we report here the primary amino acid sequence of sillucin.

2. Methods

The production, isolation and purification of the antimicrobial peptide were done by the methods in [1,3]. Purity was judged by polyacrylamide gel electrophoresis and by N-terminal analyses of the intact carboxymethylated peptide by the automated Edman procedure. Purified peptide, 2 μmol, were carboxymethylated as in [4]. The extent of modification was assessed by amino acid analyses and was found to be essentially quantitative.

Tryptic and carboxypeptidase Y [5] digestions were done on the reduced, carboxymethylated peptide. Tryptic fragments were isolated from DEAE–Sephadex equilibrated with 10 mM Tris–HCl (pH 8.2) using a linear salt gradient from 0–0.2 M NaCl.

Automated sequencer degradations of the intact R[^{14}C]CM-peptide or RCM-peptide and of the tryptic fragments were done on a Beckman sequencer model 890B (updated) using essentially the peptide program in [6]. Horse heart apocytochrome *c* (2-3 mg/run), isolated as in [7], or polybrene (3 mg/run) [8,9] was included in each run to reduce extractive losses of the peptide. Phenylthiohydantoin derivatives were analyzed by TLC [10], GLC [11] or by back hydrolysis with 55% HI at 130°C for 24 h. Amino acid analyses were performed on a Beckman amino acid analyzer model 119.

3. Results and discussion

The separation of the tryptic fragments of the carboxymethylated peptide on DEAE–Sephadex is illustrated in fig.1. Excellent separation was achieved with the dodecapeptide, T-2 fragment, eluting only upon addition of 1 M NaCl. The composition, N-terminals, and recovery yields of each tryptic peptide are shown in table 1. T-4, a tripeptide, contained no Lys or Arg, and therefore was assigned as the carboxyterminal fragment.

The carboxyterminal sequence, as well as the overlap of T-3 and T-4, were determined by degradation with carboxypeptidase Y. Automated Edman degradation on the intact RCM-peptide through 16 cycles established the sequence of the N-terminal portion of

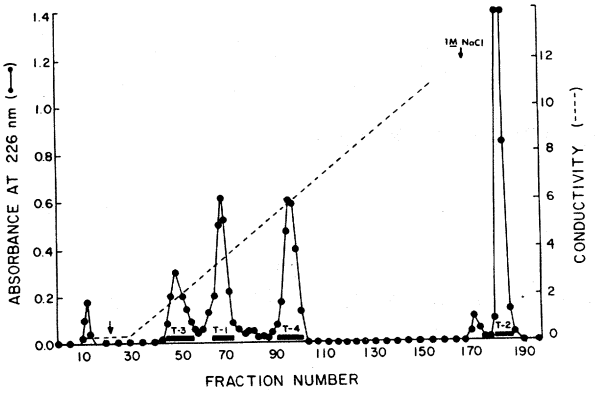

Fig.1. Elution profile of the separation of the tryptic peptides of sillucin on DEAE–Sephadex (0.01 M Tris–HCl (pH 8.2) with linear salt gradient from 0–0.2 M NaCl). The pooled fractions are designated by the bars under each peak.

the modified peptide and allowed the alignment of the tryptic peptides (T-1–T-2–T-3–T4). The sequence of residues 11–22 was established by an automated sequencer run of T-2 through 10 cycles. The tryptic pentapeptide, T-3, was sequenced through 4 cycles by automated Edman degradation in the presence of apocytochrome *c*.

The complete amino acid sequence of the *S*-carboxymethylated antimicrobial peptide is shown in fig.2 and accounts for the amino acid composition and the mol. wt 3400, estimated by analytical ultracentrifugation, reported [3].

The amino acid sequence indicates the possibility

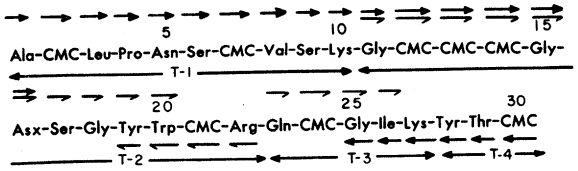

Fig.2. The complete amino acid sequence of sillucin. (→) Designated residues identified by automated sequencer runs on the entire carboxymethylated peptide. (⇀) Indicates sequencer run on the isolated tryptic peptide. (←) Denotes residue(s) identified by digestion with carboxypeptidase Y on the intact modified peptide and (↼) on the isolated tryptic fragments.

Table 1
Amino acid composition of the tryptic peptides of carboxymethylated antimicrobial peptide from *M. pusillus*

Amino acid	Total	T-1	T-2	T-3	T-4
Asp	2	1.0 (1)	1.1 (1)		
Thr	1				1.1 (1)
Ser	3	1.9 (2)	1.0 (1)		
Glu	1			1.0 (1)	
Pro	1	1.1 (1)			
Gly	4		2.9 (3)	1.0 (1)	
Ala	1	1.0 (1)			
Cys[a]	8	1.5 (2)	3.7 (4)	0.7 (1)	0.7 (1)
Val	1	0.9 (1)			
Ile	1			0.9 (1)	
Leu	1	0.9 (1)			
Tyr	2		1.0 (1)		0.9 (1)
Lys	2	1.1 (1)		1.1 (1)	
Arg	1		1.0 (1)		
Trp	1		0.4 (1)		
Total residues	30	(10)	(12)	(5)	(3)
Sequence position		1-10	11-22	23-27	28-30
% Yield		97	82	91	90
NH_2-terminus		Ala	Gly	Gln	Tyr

[a] Determined as carboxymethylcysteine

of only one acidic residue at position 16. This explains the cathodic electrophoretic migration of sillucin even at basic conditions (pH ~10).

Finally, the thermal stability and the inaccessibility of the tryptophan residue to formylation under non-reducing conditions reflects the compact structure imposed by the 4 disulfide bridges present in the native peptide [3].

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References

[1] Somkuti, G. A. and Walter, M. M. (1970) Proc. Soc. Exptl. Biol. Med. 133, 780–785.

[2] Somkuti, G. A. (1973) J. Dairy Sci. 56, 639.

[3] Somkuti, G. A. and Greenberg, R. (1979) Dev. Indust. Microbiol. 20, in press.

[4] Crestfield, A. M., Moore, S. and Stein, W. H. (1963) J. Biol. Chem. 238, 622–627.

[5] Hayashi, R., Moore, S. and Stein, W. H. (1973) J. Biol. Chem. 248, 2296–2302.

[6] Hermodson, M. A., Ericsson, L. H., Titani, K., Neurath, H. and Walsh, K. A. (1972) Biochemistry 11, 4493–4502.

[7] Bonicel, J., Bruschi, M., Couchoud, P and Bovier-Lapierre, G. (1977) Biochimie 59, 111–113.

[8] Tarr, G. E., Beecher, J. F., Bell, M. and McKean, D. J. (1978) Anal. Biochem. 84, 622–627.

[9] Klapper, D. G., Wilde, C. E. and Capra, J. D. (1978) Anal. Biochem. 85, 126–131.

[10] Inagami, T. and Murakami, K. (1972) Anal. Biochem. 47, 501–504.

[11] Pisano, J. J., Bronzert, T. J. and Brewer, B. H., jr (1972) Anal. Biochem. 45, 43–59.